ANTIBIOTICS MONOGRAPHS | NO. 9

Penicillin

Harold L. Hirsh and Lawrence E. Putnam

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FOREWORD

For 15 years, doctors have been treating patients with penicillin. Many physicians in practice today do not know what illness can be like without penicillin, and older physicians have difficulty recalling the wards full of patients with pneumonia, scarlet fever, empyema, cellulitis, osteomyelitis, and syphilis, and the morgue full of patients who died from meningitis and endocarditis.

During these 15 years, several highly effective antibiotics have been introduced, but penicillin still remains supreme in several respects: (1) several microorganisms of great importance from the standpoint of human infections, pneumococci, group A hemolytic streptococci, and gonococci, are rapidly killed by smaller amounts of penicillin than of any other antibiotic. (2) After 15 years of using penicillin, strains of those organisms that are resistant to this antibiotic have not appeared in human beings. (3) The difference between the bacteriostatic and bactericidal concentrations of penicillin for most microorganisms is smaller than in the case of any other antibiotic. (4) Penicillin is more effective than any other antibiotic in the treatment of individual cases of syphilis, and its effectiveness in this disease is responsible in great part for the rapid strides that have been made in bringing the disease under control. (5) Tremendous doses of penicillin can be used, when necessary, without ill effect. Side reactions to penicillin have been virtually absent, with the exception of hypersensitivity reactions. Even these are rarely serious if proper precautions are taken.

Penicillin has justified its early promise; it is truly a remarkable drug. It is interesting to speculate as to what would have happened if some other more toxic antibiotic had been discovered prior to the discovery of penicillin. Would investigators have been stimulated to look for others, or would the entire idea of antibiotics have been abandoned? Thus, penicillin is extremely important from a historical standpoint also.

Both Dr. Hirsh and Dr. Putnam participated in investigations on the effectiveness of penicillin beginning soon after the introduction of the antibiotic into this country. They were especially interested in ways of making penicillin more effective by improving the preparations used and the methods of administration. This led them into studies on absorption and excretion and the bacteriological control of therapy which have stood them in good stead in the preparation of this monograph. For the past few years, the authors have been engaged in the practice of medicine and have been teaching in the area

of infectious diseases in their respective medical schools. These activities have lent an intensely practical slant to this book.

The authors have wisely chosen to limit the scope of their monograph. After a concise historical introduction, they have confined themselves to the consideration of the action of the antibiotic upon microorganisms and upon man. Because each of the authors has, in the past, spent some time with the United States Food and Drug Administration, they are uniquely qualified to discuss the various preparations of penicillin that are available to the practicing physician. The resulting chapter is an authoritative source for such information, which will be valuable to manufacturer and clinician alike. The discussion of absorption and excretion is competently handled with a minimum of words. The remainder of the book is a practical treatise on the management of infections in which penicillin is or may possibly be of value, with directions as to dosages and methods of administration. The introductions to each section, in which the characteristics of the disease are given, will be of great value to the physician who uses this book as a manual of therapy. All who read it will find it is a concise, up-to-date, authoritative monograph, which should be a practical guide for many years.

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PREFACE

Penicillin has now been available for 15 years, and its usefulness and limitations are well established. It is remarkable that although it was the first antibiotic to be used extensively clinically, and although many others have been discovered since, it still has many advantages over the others. As a matter of fact, except for its somewhat limited scope of action and occasional hypersensitivity reactions, it is among the most useful of the antibiotics. It has the additional advantage of being administered by injection with the least amount of discomfort, so that its administration is certain.

The purpose of this monograph is not to give the differential choices of antibiotics in any particular condition but, rather, to delineate the usefulness of penicillin and in particular to show the dosage schedules of the various available preparations.

The recommendations advanced are based on personal experience and investigation, plus a consensus of opinion from a survey of the literature. Over the years, thousands of references have accumulated. For the purpose of this monograph, only a selected few that best exemplify the spirit of this text are listed.

The authors greatly appreciate the extensive help given by W. A. Randall, former Director of Research of the Division of Antibiotics of the United States Food and Drug Administration, who, prior to his untimely death, contributed a great deal of time and effort to the preparation of the first draft of this monograph. Thanks also are due to Mrs. Nell Draught, Mrs. H. L. Hirsh, and Mr. B. B. Boeckman for their dedicated assistance in the preparation of the manuscript.

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Chapter I History of Development and Commercial Production

The contamination in 1928 of a culture plate by spores of a species of *Penicillium* was the beginning of the study of penicillin.[1] It was indeed fortunate that the culture plate contained colonies of staphylococci that were susceptible to the antibacterial action of one of the then unknown products of the mold. Otherwise, who knows how the development of modern chemotherapy might have been delayed or altered?

The original culture plate that led to the discovery of penicillin has been preserved in Sir Alexander Fleming's laboratory at St. Mary's Hospital in London. Although originally classified as *Penicillium rubrum,* the mold was later shown by Thom, an outstanding American mycologist, to be *Penicillium notatum,* a species closely allied to *Penicillium chrysogenum.*

It was just before the beginning of World War II, in July, 1939, that Chain and Florey began their first experimental work on penicillin. When England entered the war in September, 1939, penicillin was still a laboratory curiosity produced only by certain *Penicillium* strains, and then only inconsistently and in small amounts (2 to 5 units/ml.).

Through the efforts of Florey, Chain, Heatley, and Abraham during the next few years, penicillin was produced and eventually partially purified to the point where 1 part of the amorphous yellow powder mixed with 30 million parts of medium was sufficient to inhibit the growth of staphylococci. Yet at this stage, the crude amorphous penicillin was only about 10 per cent pure. In addition, it required nearly 100 liters of the mold brew to obtain enough penicillin with which to treat 1 patient for one day. The mold was first grown in flasks and later in large vessels. Actually, bedpans were used by the original group of investigators for growing cultures of *P. notatum.* In those days there was so little penicillin available that it was frequently recovered from the urine of a patient under treatment, so that relatively pure penicillin could be readministered to the same or another patient. Case 1 in

the 1941 report from Oxford was that of a policeman who was suffering from a severe mixed staphylococcic and streptococcic infection. He was treated with penicillin, some of which had been recovered from urine. It is said that an Oxford professor referred to penicillin as a remarkable substance, grown in bedpans and purified by passage through the Oxford police force.[2]

Because of the War, British industry was unable to produce sufficient penicillin to meet the demand. In the summer of 1941, Florey and Heatley came to the United States and successfully stimulated American industry to manufacture this much-needed drug. Certain pharmaceutical houses produced penicillin by the bottle method, while others rapidly developed techniques for growing enormous quantities of the drug by deep-tank fermentation.

A great deal of effort was put into the problems of increasing yields in the fermentation process and of improving extraction methods. Consequently, in a relatively short time, great quantities of the drug were being produced. In 1941 there was not enough penicillin in the United States to treat a single patient. In 1942 there were probably insufficient quantities to treat 100 patients. By April, 1943, approximately 200 patients had been treated,[3] and by September, 1943, there was enough penicillin to supply the needs of our Armed Forces and those of our allies.

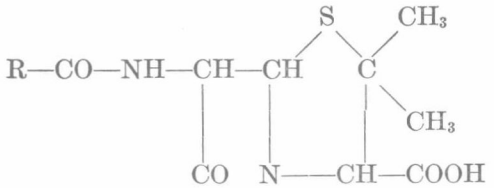
In the early stages, the drug was of low purity. Its potency ranged from 100 to 200 units/mg. (10 to 15 per cent pure). With increased production there was an increase in the purity of the penicillin. The original dark brown amorphous powder gradually became lighter in color. After controlled studies showed that the pain produced by intramuscular injections of penicillin solutions was inversely proportional to their purity,[4] the minimum requirements of potency were raised. By 1946 pure white crystalline penicillin was being produced commercially.

Production, which amounted to a few hundred million units a month in the early days, increased with great rapidity, until by January, 1949, more than 8000 billion units of the drug were being produced monthly. In January, 1950, production increased to 16,000 billion units, and a few months later, to 20,000 billion units. The annual production for human use from 1950 to the present was as follows: 1950, 222 trillion units (148 tons); 1951, 324 trillion units (216 tons); 1952, 350 trillion units (233 tons); 1953, 375 trillion units (250 tons); 1954, 450 trillion units (300 tons); 1955, 328 trillion units (219 tons); 1956, 449 trillion units (299 tons); 1957 (estimated), 562 trillion units (375 tons).

While originally penicillin was thought to be a single substance, it was soon discovered that there were at least five naturally occurring types of the drug. The penicillin produced in England from 1939 to 1941 consisted of a product containing varying amounts of what were later called penicillins I, II, and III by the English workers. In this country they were called penicillins

F, G, and X, respectively. Later other types, such as dihydro F, K, O, and V, were discovered. With the benefit of data accumulated by Sheehan and Henery-Logan[5] in their successful practical synthesis of penicillin V, it is probable that other new types will be forthcoming. All such forms are covered by the regulations promulgated under the Federal Food, Drug, and Cosmetic Act,[6] which states that "each of the several antibiotic substances (e.g. penicillin F, penicillin G, penicillin X) produced by the growth of *P. notatum* or *P. chrysogenum,* and each of the same substances produced by other means, is a kind of penicillin."

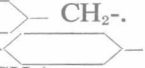
The following structural formula of penicillin contains the unusual beta-lactam thiazolidine configuration:



The different penicillins so far examined have the same general structure and vary only in the nature of the side chain, R, as follows:

In penicillin F (I), R is CH_3-CH_2-CH=CH-CH_2.

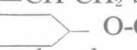
In penicillin G (II), R is  CH_2-.

In penicillin X (III), R is HO  CH_2-.

In penicillin K (IV), R is CH_3-$(CH_2)_6$-.

In penicillin dihydro F, R is CH_3-$(CH_2)_5$-.

In penicillin O, R is CH_2=CH-CH_2-S-CH_2-.

In penicillin V, R is  O-CH_2-.

Penicillin X has been found to be more effective than penicillin G in certain diseases.[7] However, penicillin G was found to be more effective than penicillin X in other diseases,[8] and since the methods of manufacture that produce the greatest amounts of penicillin give the largest yield as penicillin G, penicillin X is no longer manufactured. Penicillin K is rapidly excreted from the body and consequently has poor therapeutic efficacy. Penicillin F and dihydro F are therapeutically active but are not produced at the present time. Limited amounts of penicillins other than G may exist in preparations labeled as penicillin G.

Before the various types of penicillin had been classified and separated, many salts of penicillin had been prepared and used for clinical experimental purposes. Among these, it was shown that the calcium, magnesium, sodium, strontium, potassium, lithium, and ammonium salts were effective in the treatment of gonorrhea.[9] Aluminum penicillin is also used clinically.

The sodium and the potassium salts have been crystallized and are the

principal types of soluble penicillin. The calcium salt has never been crystallized, but, because it is nonhygroscopic, it was used extensively in combination with oil and beeswax from 1944 to 1948.[10]

Crystalline penicillin G as the sodium or potassium salt is extremely stable. These salts may be held at temperatures of 100 C. for four days without significant loss of potency. The expiration date of five years from the date of manufacture, which appears on the labels of crystalline penicillin, is conservative. However, once in solution, the drug is not stable and must be refrigerated. It is inadvisable to use unbuffered solutions more than three days old unless they can be assayed to assure full potency. Buffered solutions of penicillin G are somewhat more stable and may be stored up to seven days in the refrigerator.

Procaine penicillin was first prepared accidentally in an attempt to mitigate the pain of injection by adding procaine to solutions of penicillin. The precipitate that formed was studied, and it was noted that its insolubility delayed absorption and excretion. In comparable dosages, procaine penicillin produced blood levels that were at least as well sustained as those with any previous penicillin preparations. In addition, suspensions in water were easy to handle and eliminated the oil and wax used in the older preparation.

Crystalline procaine penicillin G is not so stable to heat as crystalline sodium or potassium penicillin G. It decomposes at temperatures between 70 and 80 C. It is relatively stable in aqueous suspension at room temperature, but should not be exposed to temperatures of 25 to 30 C. for extended periods of time. Aqueous suspensions of procaine penicillin G have an expiration date of 12 to 24 months at temperatures less than 25 C. (77 F.). Since, in most climates in this country, consistent room temperatures of 77 F. or less cannot be relied on, it is advisable to refrigerate suspensions of procaine penicillin G. This will help maintain the potency and proper physical characteristics. These preparations contain a preservative and may also have buffer substances and dispersing or stabilizing agents. These substances may materially affect the physical characteristics of the preparation when not refrigerated. At a temperature of 80 F. for five months, aqueous suspensions of procaine penicillin G have been observed to thicken and set like cement, even though the potency was unaffected.

In 1953, penicillin O was introduced on the American market. This preparation, allylthiomethyl penicillin, has been found useful in many instances in the treatment of patients sensitive to penicillin G.

In order to have an insoluble depot form of penicillin O, the 2-chloroprocaine salt has been prepared.

Other salts of penicillin G with organic bases, which have different solubilities, have been prepared. These have been utilized for achieving prolonged blood concentrations on injection and for preparing stable oral sus-

pensions. They include benzathine penicillin G (*N, N'*-dibenzylethylenediamine dipenicillin G), *l*-ephenamine penicillin G, dibenzylamine penicillin G, and hydrabamine penicillin G.

By esterifying the acid function of penicillin G, the diethylaminoethyl ester of penicillin G was prepared. The hydriodide salt of this ester is used for intramuscular injection. Although it is biologically inactive, the ester hydrolyzes readily after injection and produces relatively high tissue concentrations, particularly in the lung. However, this form is not used in the United States today because of an inordinate number of fatal reactions after its use.

In 1955, a new biosynthetic penicillin became available. Phenoxymethyl penicillin, or penicillin V, as it is called, has the unique property of being very stable in gastric acid. This makes it particularly suitable for oral medication. It gives blood concentrations approximately double those obtained from a comparable oral dose of penicillin G and is believed to be more dependable than penicillin G. Of interest is the fact that phenoxymethyl penicillin was discovered in 1948 by Behrens et al,[12] but its resistance to acid degradation was not known until 1953, when a group of workers in Austria came upon this property[13, 14] (see Chapter XII).

To date, the following milestones in the history of penicillin therapy may be enumerated. (1) The discovery of penicillin in 1928 by Sir Alexander Fleming. (2) The use of penicillin by Chain, Florey, and others in 1939-1941 for the treatment of diseases in man. (3) The development of a prolonged acting preparation by Romansky in 1944. (4) The use of the relatively insoluble and somewhat anesthetic procaine penicillin in December, 1948. (5) The marketing in April, 1952, of benzathine penicillin G, a salt of penicillin that is so insoluble that intramuscular injections of 1.2 million units provide measurable blood concentrations for approximately four weeks.[15] This preparation simplifies the prophylaxis of rheumatic fever. (6) The introduction in 1955 of acid-stable penicillin V for reliable oral therapy.

Chapter II Antimicrobial Activity

Penicillin is active primarily against the gram-positive organisms. In vitro, pneumococci, streptococci, many staphylococci, and certain of the clostridia are susceptible to its action. In the gram-negative group, meningococci and gonococci are susceptible to penicillin; *Hemophilus influenzae* and *Salmonella typhosa* occupy an intermediate position, while *Escherichia coli, Salmonella paratyphi, Salmonella enteritidis, Aerobacter aerogenes,* and *Pseudomonas aeruginosa* are resistant.

Resistance of gram-negative organisms is one of degree only, since penicillin has some activity against *Sal. typhosa* and *Proteus* species.

Fungi are also generally resistant to penicillin, except certain of the *Actinomyces*. The resistance of certain of the gram-negative organisms is associated with their ability to produce penicillinase, an enzyme that inactivates penicillin. A relatively large number of organisms, including the fungi, have the capacity to produce this substance.[16] Gram-positive organisms also are capable of producing penicillinase and is one of the means by which these organisms may develop resistance to penicillin.[17] Penicillinase is of value in sterility test procedures and in blood culture studies, where it is essential to inactivate penicillin before attempting to isolate suspected organisms. The enzyme is of clinical use in treating patients having allergic reactions to penicillin.

The antibacterial activity of penicillin in vitro is not appreciably affected by the presence of blood and serum. In addition to its activity against vegetative forms of organisms, penicillin is sporicidal in relatively low concentrations. It also has marked treponemicidal activity.

Penicillin has been shown to prevent or delay death of mice and chick embryos infected with psittacosis virus.[18] Other members of the psittacosis-lymphogranuloma group are also inhibited by its action.[19] Organisms of the genus *Leptospira* have been shown to be susceptible to the action of penicillin.[20] All of 29 strains of *Leptospira* tested in vitro were either partly or completely

inhibited by relatively low concentrations of the drug. Although there was considerable variation in the susceptibility of the species tested, most of the common pathogenic organisms of the *Leptospira* group were completely inhibited by 0.5 unit/ml. or less. A variation in resistance was shown within the species *Leptospira icterohaemorrhagiae*. None of the organisms tested in these studies produced penicillinase.

In general, penicillin is not active against the protozoa. Cultures and cysts of *Endamoeba histolytica* are not affected by penicillin in concentrations of 5000 to 10,000 units/ml.[21] Some evidence of temporary symptomatic improvement has been reported in amebiasis during penicillin administration. However, stools have remained positive during treatment and symptoms recurred promptly after cessation. Combinations of penicillin with other drugs have given variable results, but there appears to be no valid reason for using penicillin in amebic infections except where it appears that secondary infection with penicillin-sensitive organisms has occurred.

The early studies of the effect of penicillin on the tubercle bacillus were done with relatively crude penicillin preparations, but it appeared that concentrations of 40 units/ml. did not inhibit the growth of this organism. More recent studies using crystalline sodium penicillin G have shown that human, bovine, and avian types of the tubercle bacillus are inhibited by concentrations varying from 1 to more than 200 units/ml.[22] Bovine and avian strains of the tubercle bacillus were more sensitive to penicillin than were human strains. In any case, penicillin is of no value in the treatment of tuberculosis except in those circumstances where a tuberculous patient has developed or is exposed to an infection due to a penicillin-sensitive organism.

The literature that has accumulated showing the activity and inactivity of penicillin for microorganisms is voluminous. This antimicrobial activity is summarized in table I, where the range of sensitivities is shown along with the concentrations of penicillin to which the majority of strains tested are sensitive.[23]

In attempts to broaden the antimicrobial activity of penicillin, a number of combinations with other drugs have been studied. The greater effectiveness of sulfonamides and penicillin used concomitantly, in contrast to either agent alone, in the treatment of pneumococcal meningitis was first pointed out in 1944 by Waring and Smith.[24] Hobby and Dawson[25] and Garrod[26] first carried out experiments showing antagonism between sulfonamides and penicillin. In 1946, Hobby and Dawson[27] reported on the combined action of penicillin with sulfadiazine and showed that the results depended on (1) the concentration of each bacteriostatic agent present, (2) the number of organisms, (3) the environmental conditions allowing growth of the organisms, (4) the degree of susceptibility of the organisms to the antimicrobial agents concerned, and (5) the individual species of the organisms involved.